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VALIDATED SPECTROPHOTOMETRIC QUANTITATION OF LEVOFLOXACIN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A novel, simple, accurate and precise Zero order derivative spectroscopic method was developed and validated for the estimation of Levofloxacin in bulk and Pharmaceutical dosage forms and has an absorption maximum at 293.6 nm in 0.1N Sulphuric acid. The Linearity was found to be in the concentration range of 5-30µg/ml and the correlation coefficient was found to be 0.999 and it has showed good linearity, reproducibility, precision in this concentration range. The regression equation was found to be Y = 0.019 X + 0.003. The % recovery values were found to be within 99.14 -100.16 % showed that the method was accurate. The LOD and LOQ were found to be 0.3041 and 0.9123µg/ml, respectively. The % RSD values were less than 2. The method has been validated according to ICH guidelines for linearity, accuracy, precision, robustness, ruggedness. Limit of detection and limit of quantitation. Proposed method was successfully applied for the quantitative estimation of Levofloxacin in bulk and pharmaceutical dosage form.

KEYWORDS: Levofloxacin, Zero order derivative Spectroscopy, 0.1N Sulphuric acid, Linearity, Precision, Reproducibility, and Accuracy.

INTRODUCTION

Levofloxacin (LVFX) is a synthetic fluoroquinolone antibacterial agent that inhibits the supercoiling activity of bacterial DNA gyrase, halting DNA replication. It is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis.[1,2]

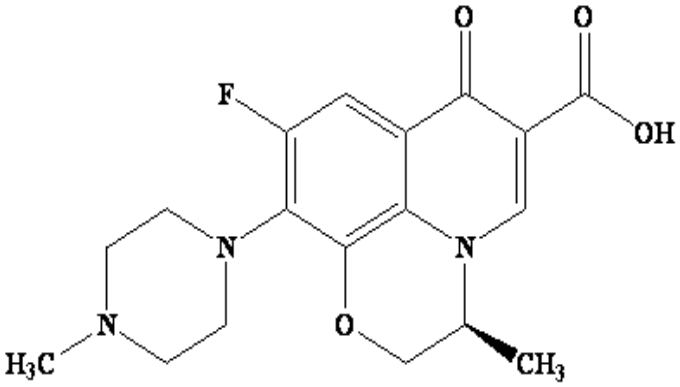

Figure 1: Chemical structure of Levofloxacin.

Levofloxacin is chemically (S)-9-Fluoro-2,3-dihydro-3-methyl- 10-(4-methyl-1-piperazinyl)-7 oxo-7H-pyrido(1,2,3-de) -1,4 benzoxazine-6-carboxylic acid, is a new quinolone antimicrobial agent which exhibits broad-spectrum in vitro bactericidal activities against gram-positive and gram-negative aerobes[3]. It has a molecular formula of $C_{18}H_{20}FN_3O_4$ and molecular weight of 361.368g/mol. It has the structural formula (Fig.1). Levofloxacin is a yellowish white powder. which is freely soluble in Glacial acetic acid, chloroform, sparingly soluble in water. Brand name of Levofloxacin is Levaquin.

Literature Survey revealed that the drug has been estimated by UV-Spectrophotometric,[3-23] RP- HPLC.[24-35] and HPTLC.[36] method has been reported so far.

The aim of present work was to develop and validate a novel, rapid, simple, precise, and specific Zero order derivative UV-Spectrophotometric method for estimation of Levofloxacin in its bulk and tablet dosage form.

MATERIALS AND METHOD

Instrument

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken on analytical balance.

Chemicals

Levofloxacin pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form Levoflox 10 Tablets labelled claim 500 mg were purchased from local pharmacy manufactured by Cipla Ltd.

Solvent

0.1N Sulphuric acid (prepared by dissolving 2.75ml in 1000ml of distilled water).

Selection of analytical wavelength

Appropriate dilutions were prepared for drug from the standard stock solution and the solution was scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained were derivatized from Zero order method. It shows maximum absorbance at 293.6 nm was shown in Fig.2 and Zero order overlain spectra of Levofloxacin at 293.6 nm were shown in Fig.3.

Preparation of Standard stock solution

Accurately weigh 100mg of Levofloxacin was transferred into 100ml volumetric flask and diluted with 0.1N Sulphuric acid up to the mark. From this pipette out 10ml into 100ml volumetric flask and diluted with 0.1N Sulphuric acid up to the mark, from this solution pipette out 0.5, 1,1.5, 2,2.5 and 3.0ml into 10ml individual volumetric flask and add 0.1N Sulphuric acid up to the mark, this gives 5, 10, 15, 20, 25 and 30µg/ml concentrations.

Preparation of Sample solution

Twenty tablets were weighed and powdered, the tablet powder equivalent to 100mg of Levofloxacin was transferred into100ml volumetric flask then it was diluted with 0.1N Sulphuric acid and made up to mark and the solution was filtered through Whatmans filter paper no.41. From this pipette out 10 ml in a 100 ml volumetric flask and make up the volume up to the mark with 0.1N Sulphuric acid. From this solution pipette out 2 ml into 10ml volumetric flask and make up the volume with 0.1N Sulphuric acid, this gives 20µg/ml concentrations.

Method validation

The method is validated according to the ICH guidelines.

RESULTS AND DISCUSSION

Method: Zero order derivative spectroscopy

Linearity

The working standard solution were diluted serially with 0.1N Sulphuric acid to obtain the range of 5-30µg/ml. a calibration curve for Levofloxacin was obtained by measuring the absorbance at the λmax of 293.6nm and absorbance values are shown in Table.1 and

Calibration graph were presented in Fig.4. Statistical parameters like slope, intercept, coefficient of correlation, and Sandel's sensitivity were determined and presented in Table.2.

Table 1: Results of calibration curve at 293.6 nm by zero order Spectroscopy.

Sl. No.	Concentration in µg/ml.	Absorbance ± Standard deviation
1	5	0.103± 0.002828
2	10	0.198± 0.00772
3	15	0.291± 0.004382
4	20	0.390± 0.002828
5	25	0.483± 0.005477
6	30	0.582± 0.005477

Table 2: Regression parameters for Levofloxacin by zero order spectroscopy

Regression Parameters	Levofloxacin
Range	5-30µg/ml
λMax	293.6nm
Regression Equation	Y=0.019x+0.003
Slope (b)	0.019
Intercept(a)	0.003
Correlation coefficient (r2)	0.999
Sandell's Sensitivity	0.0512

Table 3: Determination of precision results for Levofloxacin at 293.6 nm by zero order derivative spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance ±SD**	% RSD	Inter-day Absorbance ±SD**	% RSD
5	0.101± 0.001	0.990	0.101± 0.001528	1.512
10	0.191±0.001528	0.80	0.195± 0.003	1.538
15	0.292±0.001528	0.523	0.290± 0.002517	0.867
20	0.389±0.001155	0.296	0.388± 0.002517	0.648
25	0.480±0.002517	0.524	0.483± 0.002082	0.431
30	0.579±0.002517	0.434	0.579± 0.003055	0.527

Table 4: Determination of accuracy results for Levofloxacin at 293.6nm by Zero order derivative spectroscopy.

Spiked levels	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Amount recovered	%Recovery ±SD**	%RSD
50	10	5	14.86	99.14±0.68	0.6858
100	10	10	19.84	99.23±0.77	0.7759
150	10	15	25.03	100.16±0.3153	0.3148

**Average of six determinations

Table 5: Ruggedness results of Levofloxacinat 293.6 nm by Zero order Spectroscopy

Analysts	Analyst-1	Analyst-2
Mean absorbance	0.290	0.293
Standard deviation	0.003633	0.003899
% RSD	1.252	1.330

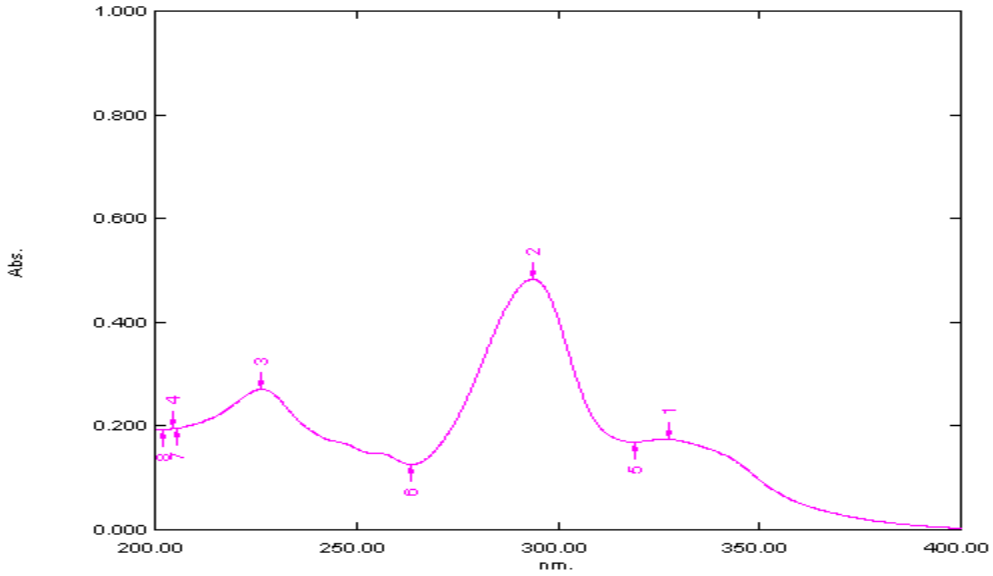

Fig. 2: Zero order spectra of Levofloxacin showing the absorbance at 293.6 nm.

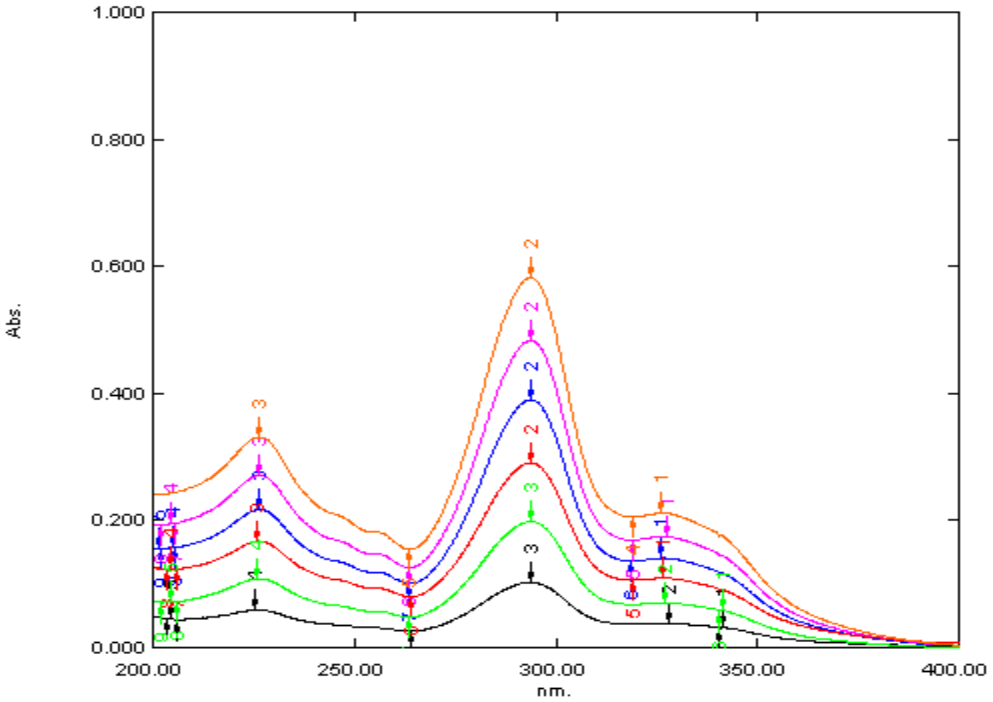

Fig. 3: Zero order overlain spectra of Levofloxacin showing absorbance at 293.6 nm.

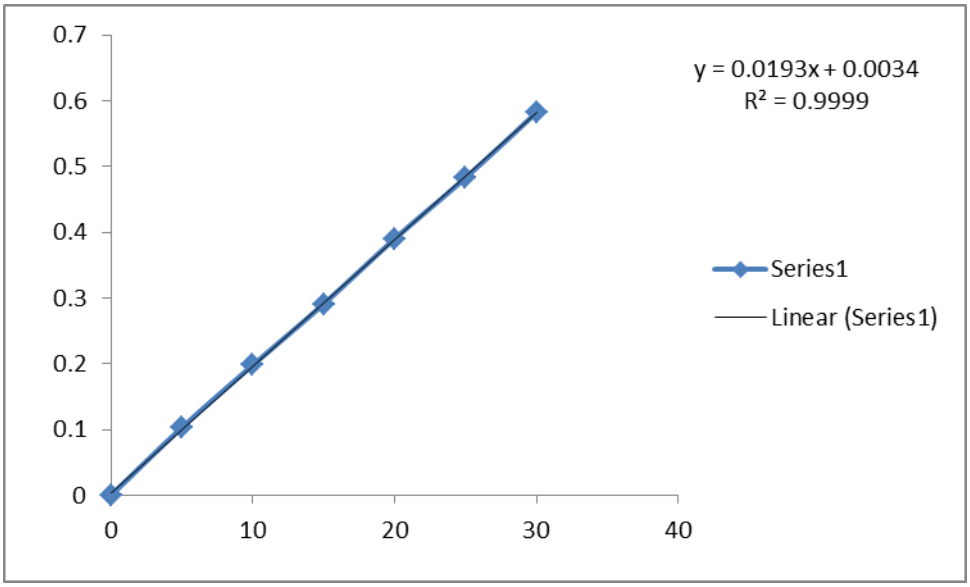

Fig. 4: Linearity curves for Levofloxacin at 293.6 nm by zero order Spectroscopy.

Precision

Precision of the method was studied as intra-day and inter-day precision. Intra-day precision was determined by analyzing the 5, 10, 15, 20, 25 and 30µg/ml concentration for three times in same day. Inter-day precision was determined by analyzing the same concentration of solution daily for three days. Precision results are shown in Table.3.

Accuracy

To assess the accuracy of the proposed method, recovery studies were carried out at three different levels i. e, 50%, 100% and 150%. In which the formulation concentration was kept constant and varied pure drug concentration. Accuracy results were shown in Table.4.

Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was found to be less than 2 were shown in Table.5.

Limit of detection and Limit of Quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the Response and slope of linearity curve. LOD and LOQ values of Levofloxacin were found to be 0.3041µg/ml and 0.9123µg/ml.

CONCLUSION

From the above it can be concluded that all validation parameters such as precision, accuracy, linearity, LOD, LOQ and Ruggedness met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed spectrophotometric method is simple, rapid, accurate, and precise and can be applied for routine analysis of Levofloxacin in bulk and its dosage forms.

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